THE RESPIRATORY METABOLISM OF EXCISED TISSUES OF WARM- AND COLD-ADAPTED FISHES

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A striking characteristic of certain arctic poikilotherms such as the arctic (or polar) cod, blackfish, sculpin and indigenous isopods and amphipods is their ability to remain active at environmental temperatures ranging around 0° C. or lower. In the case of the fish there is good reason to believe that body temperature is within a few tenths of a degree of the temperature of the water habitat (Clausen, 1934; Gunn, 1942). Polar cod are often found in sea water in ice pockets and cracks where the water temperature is below 0° C. In contrast, many poikilothermic forms living in the temperate zone are in a state of cold narcosis at such temperature (Parker, 1939). In consonance with these observations is the finding that failure of conduction occurs in the sciatic nerve of the green frog at about 5° C. (Gasser, 1931).

Recently Irving and his associates (Edwards and Irving, 1943a, 1943b; Haugaard and Irving, 1943) described a form of adaptation to temperature in certain non-hibernating poikilotherms. They showed that in these animals the effect of temperature on metabolic rate depends in part on the temperature of the environment from which the animal was taken. Thus, at temperatures below 20° C., the oxygen consumption of the sand crab (*Emerita talpoida,* Say), after allowance for differences in size, was greater in winter than in summer. Both the temperature of maximum oxygen uptake and the thermal death point (range) were lower in winter than in summer (Edwards and Irving, 1943a). This change in the properties of the biological oxidation system on long exposure to cold may be regarded as a primitive form of chemical defense, and the continued growth and activity of the sand crab during the winter indicate that it is an important adjustment of the animal to season. No such seasonal influence on the effect of temperature on oxygen consumption was found in the beach flea (*Talorchestia megalopthalma*), an air-breathing neighbor of the sand crab (Edwards and Irving, 1943b). In line with these differences, the beach flea, instead of remaining active during the winter, went into a state of apparent hibernation beneath the sand.

In the cunner (*Tautologolabrus adspersus,* Walbaum) there was some adaptation of the oxidative metabolism to season, but this was insufficient to allow the necessary physical activity to enable the cunner to remain in the summer habitat during the winter months. This view agrees with the disappearance of the cunner from the shoreline waters in winter (Haugaard and Irving, 1943).

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It has long been established that poikilotherms can be acclimatized to withstand environmental temperatures that are normally lethal (Davenport and Castle, 1896; Vernon, 1899–1900; Loeb and Wasteneys, 1912). The relationship between environmental temperature and lethal temperature has been defined more precisely in a number of acclimatization studies by Hathaway (1927), Fry, Brett and Clawson (1942), Doudoroff (1942, 1945) and Brett (1944). Earlier work has provided other examples of metabolic adaptation to temperature in poikilotherms (Battle, 1926; Britton, 1930; Barcroft, 1934; Fox, 1939) and in hibernating homoiotherms (Tait, 1922; Britton, 1930; Suomalainen, 1939). Wells (1935) demonstrated a variation of respiratory metabolism of certain fish with season, and showed that the oxygen consumption was high in the late winter months and low during the summer months. He concluded that "it seems certain that there is some adaptation to high and low temperatures in fish."

On the basis of the evidence in hand it seems reasonable to assume that the phenomenon of metabolic adaptation to temperature is a rather general one. However, no evidence is presently available as to the nature of this effect. The present work was designed to provide data on the tissue metabolism of cold- and warm-adapted forms in relation to environmental temperature, which might contribute to the elucidation of the marked tolerance for cold shown by arctic cod and provide further information as to the nature of metabolic adaptation to temperature.

Methods

The work dealing with the cold-adapted fish was performed at the Arctic Research Laboratory, Point Barrow, Alaska (Shelesnyak, 1948). The form chosen was the polar cod, *Boreogadus saida,* Lepechin (Jordan and Evermann, 1898; Jordan, 1905, 1923), which is found in large schools around the edges and in crevasses of floating and pack ice. Since the shoreline at Point Barrow is ice-free for only a short time each year, it is necessary to utilize the available time to the fullest. Accordingly, during the period of six weeks when the ice was relatively close to shore, large numbers of fish were caught and maintained in aquaria in the laboratory.[3] While the polar cod is occasionally available at other times by fishing through the ice, the obvious difficulties of extreme cold and ice thickness make procurement very difficult. The water temperature in the habitat of the fish at the time of capture varied from − 1.5 to + 2.0° C. The fish were maintained in the laboratory in aerated aquaria at temperatures ranging from − 1.0 to 0° C.

The experiments with the Golden Orfe, *Idus melanotus* (Heckel and Kner, 1858; Guenther, 1868, 1880; Buiytendijk, 1910), were all carried out in the laboratories of the Department of Physiology at Stanford University. These fish were obtained from a commercial dealer, who maintained the aquarium temperature at 25° C. for a minimum period of one week. Suitable numbers of these fish were transferred periodically to the laboratory aquarium, where they were maintained at 25° C. until used. In general, the fish had been living at a 25° C. environmental temperature for two weeks before use.

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The following procedures and methods apply to both species of fish. The fish were decapitated, and the brain and liver were rapidly excised. Since the physical properties of the tissue precluded the preparation of brain slices, the whole brain was finely minced with scissors. Liver slices were prepared by the Lucite template method (Crismon and Field, 1940) by means of a clean dry safety razor blade (Field, 1948). The moist cold box technique was used throughout (Peiss and Field, 1948; Field, 1948). Respiration was measured by the Warburg manometric method (Dixon, 1943; Umbreit, Burris and Stauffer, 1945). The gas phase was oxygen. The center wells of the respirometer flasks contained 10% KOH with Whatman No. 40 filter paper wicks. The liquid phase was Ringer-phosphate, pH 7.4 (glass electrode). Manometric measurements were made in a constant temperature bath, equipped with a refrigerator unit, over a temperature range of 0° to 25° C. At any given temperature, the water bath was maintained at ± 0.02° C. Readings were taken at intervals of 10–20 minutes, depending on the respiratory rate of the tissue at the temperature used, and were carried out for a minimum of 120 minutes. Results are expressed in terms of wet weight Q_{O_2}. Thus Q_{O_2} (wet wt.) denotes microliters of oxygen consumed, measured under standard conditions, per milligram initial wet weight of tissue per hour.

Tissue water content was determined by drying to constant weight at 103° C. The mean water content and range, in per cent, for *B. saida* were: brain, 80.5, range 79.4–82.0, 26 fish; liver, 55.2, range 48.2–70.4, 24 fish. The values for *I. melanotus* were: brain, 78.4, range 76.4–80.4, 18 fish; liver, 74.6, range 71.4–76.8, 14 fish. The large range in *B. saida* liver appears to be due to variable amounts of oil residue in the tissue.

Results

1. *Physical measurements*

The polar cod was chosen for this study for a number of reasons, chief of which were the relatively high activity of the animals at low temperature, the large number of animals available and the relative ease with which they were obtainable. The Golden Orfe was selected as the warm-adapted fish primarily because it was the most readily available fish, in large numbers, which could withstand the high environmental temperature, and which was similar in body size to the polar cod. While the cod is a marine fish and the Orfe a fresh water form, the sea habitat of the cod we used was one of low salinity, and it has been shown that this species of cod survives readily in fresh water. Table I summarizes the body weight and length data for the two species.

Table I

Body weight and length data for polar cod (B. saida) and Golden Orfe (I. melanotus)

	B. saida		*I. melanotus*	
	Weight, gms.	Length, cm.	Weight, gms.	Length, cm.
Mean	21.1	15.5	16.8	13.0
Range	9.8–32.0	10.0–20.5	7.3–25.2	8.5–16.5
Std. dev.	4.7	2.2	3.8	1.9
No. fish	90	90	94	94

For both species of fish, the wet weight of the brain was very similar, averaging about 120 milligrams. There was, however, a wide discrepancy in the size of the liver. In the Orfe, this organ was rather diffuse, and was made up, for the most part, of three long, slender lobes. The average weight for the liver of the Orfe was of the order of 200–300 mg. The liver of the polar cod, in contrast, was large in comparison to the total weight of the whole fish. In most cases it weighed from 750–900 mg., but it was not uncommon to find a liver weighing more than a gram in a fish whose total body weight was on the order of 20 grams.

2. Time course experiments

An extensive series of experiments were made to determine the effect of time on oxygen consumption over the temperature range 0–25° C. In the case of brain and liver tissue of the Orfe, oxygen consumption was a linear function of time at all temperatures for 180–240 minutes, which was the maximum time of observation. The data for the polar cod were more variable with regard to temperature. These data are shown in Table II.

TABLE II

Duration of steady states of oxygen consumption of polar cod brain and liver at graded temperatures. Periods marked with asterisks are those in which oxygen consumption was still constant when readings were terminated

Temperature, °C.	Brain mince	Liver slices
0	300 min.*	360 min.*
5	300 min.*	360 min.*
10	240–270 min.	360 min.*
15	170–210 min.	200–240 min.
20	90–140 min.	120–150 min.
25	10– 50 min.[a]	20– 60 min.[a]

[a] At this temperature there was wide variation in the constancy of oxygen consumption with time. This was more pronounced with the brain mince. See text for further discussion.

It can readily be seen that as the temperature is increased the period of time during which oxygen consumption is constant decreases, this effect being more pronounced with the brain mince. At 25° C. it was possible to obtain satisfactory readings for at least 40 minutes in most preparations of liver slices. However, when brain mince was used at this temperature, many of the runs resulted in die-away curves from zero time. It was possible to obtain 3 readings at 10 minute intervals, during which oxygen consumption was constant, in only about 50 per cent of the runs. Thus, it was considered advisable to represent the oxygen consumption of polar cod brain mince at 25° C. by two figures, one representing the mean value for all determinations on the basis of a one hour period, and the other representing the mean value calculated from those runs in which oxygen consumption was constant for 30 minutes or more. This will be pointed out again when Table III and Figure 1 are discussed.

3. Experiments with polar cod and Golden Orfe brain mince

The oxygen consumption of brain mince from polar cod (*B. saida*) and Golden Orfe (*I. melanotus*) was determined at temperatures ranging from 0°–25° C., at

5° intervals. Approximately 100 fish of each species were used, and from 15–30 determinations were made for a given temperature. These data, together with certain statistical measures, are shown in Table III.

The upper figure at 25° C. for *B. saida* is taken from the portion of the oxygen uptake curves of those determinations that were constant for 30 minutes or more. The lower figure represents the overall mean for all determinations over a period

TABLE III

Respiration of B. saida and I. melanotus brain mince at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.267	.356	.523	.794	1.128	1.649* 1.252
Range	.217– .309	.309– .418	.418– .666	.732– .855	.928– 1.382	.817– 1.513
Standard deviation	.027	.029	.064	.035	.116	.188
Number runs	30	26	30	26	26	18
Number fish	14	21	15	17	15	8

I. melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.078	.114	.137	.203	.361	.602	.926	1.370
Range	.051– .109	.094– .133	.110– .165	.176– .231	.277– .480	.504– .707	.700– 1.181	1.131– 1.702
Standard deviation	.017	.014	.015	.017	.048	.056	.132	.162
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

* This value obtained if first 30 minutes are used for calculating (see text).

of one hour. All other figures are based on readings taken from steady state oxygen uptake. Figure 1 illustrates the data graphically. The upper solid circle at 25° C., is taken from the upper figure in Table III, and the lower solid circle from the lower figure in Table III.

When the logarithm of oxygen consumption, expressed as Q_{O_2}, is plotted against temperature in degrees centigrade, several interesting features are apparent from the curves obtained. Neglecting the 25° C. point for *B. saida,* the cold-adapted fish (solid circles), log oxygen consumption appears to be a rectilinear function

of temperature. The dotted line represents the line of "best fit" as calculated by the method of least squares (Snedecor, 1946). The regression equation is:

$$\text{Log } Q_{O_2} = -0.59 + 0.0318t.$$

The Q_{10}, calculated from this equation, is 2.08.

The curve for *I. melanotus* (open circles) appears to be rectilinear over the range 10–25° C., although it is not possible to decide just where the slope starts to change. The regression line for the rectilinear portion of the curve (dotted line) is:

$$\text{Log } Q_{O_2} = -0.81 + 0.0386t.$$

The Q_{10} calculated therefrom is 2.43.

The most striking aspect of the curves in Figure 1 is the sharp break in the lower curve below 10° C. Such a break indicates an increase in the Q_{10} for the values below 10° C., and does not appear in the curve for the polar cod (Table V). The possible significance of this difference will be discussed later.

4. Experiments with polar cod and Golden Orfe liver slices

The overall data, and certain statistical measures, for the oxygen consumption of liver slices of polar cod and Orfe are shown in Table IV. Figure 2 illustrates the data graphically as with the brain mince experiments. The curves resulting from a plot of log oxygen consumption against temperature reveal the same pattern

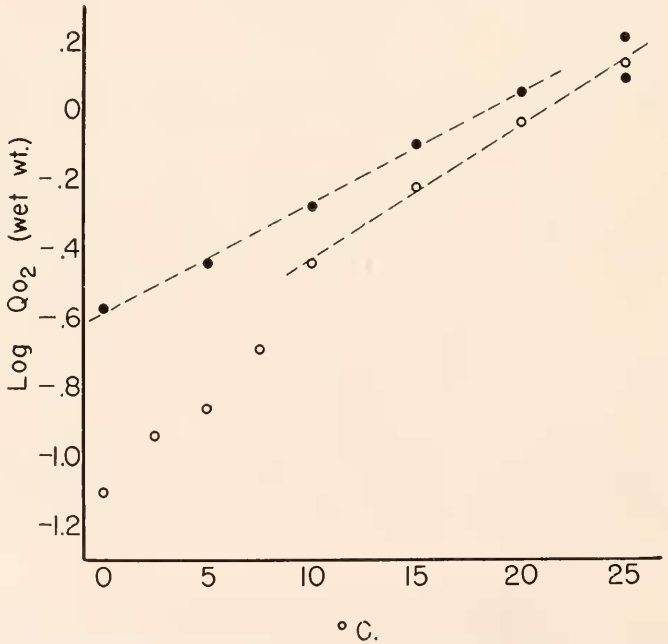

FIGURE 1. Logarithm of oxygen consumption in brain mince of *Boreogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

TABLE IV

Respiration of B. saida and I. melanotus liver slices at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.137	.186	.269	.413	.604	.859
Range	.108–.169	.159–.237	.204–.346	.372–.475	.530–.650	.340–1.093
Standard deviation	.016	.020	.040	.017	.042	.248
Number runs	30	26	34	26	26	18
Number fish	14	21	15	17	15	8

I. Melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.057	.077	.117	.171	.232	.369	.552	.792
Range	.043–.071	.056–.104	.085–.142	.141–.193	.200–.261	.297–.433	.411–.640	.643–.914
Standard deviation	.010	.017	.016	.017	.017	.040	.066	.080
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

as that seen above with brain mince (Fig. 1). The regression equation for the cold-adapted polar cod (solid circles) is:

$$\text{Log } Q_{O_2} = -0.88 + 0.0324t.$$

And the Q_{10} calculated from this equation is 2.11. The regression equation for the warm-adapted Golden Orfe (open circles) is:

$$\text{Log } Q_{O_2} = -0.97 + 0.0352t$$

with a Q_{10} equal to 2.25 over the range 10–25° C. Again, the curve for the cold-adapted form (*B. saida*) appears to be rectilinear over the entire temperature range 0–25° C., while that for the warm-adapted form (*I. melanotus*) is rectilinear only over the range 10–25° C. As in the experiments with brain mince from these two species, the Q_{10} increases sharply for the warm-adapted fish below 10° C. (Table V). Thus, although the respiratory rate of the liver slices is of the same order of magnitude at temperatures of 10° C. and higher, the arctic cod liver respires at progressively higher rate, relative to that of Orfe liver, as the temperature decreases from 10° C. to 0° C. In both brain mince and liver slice, the respiratory rate of

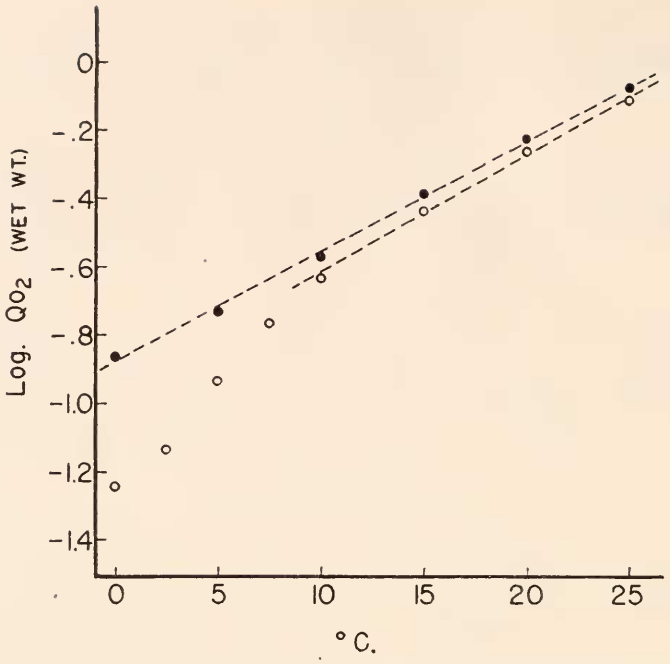

FIGURE 2. Logarithm of oxygen consumption in liver slices of *Borcogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

the tissues from the polar cod is two to three times that of the respective tissues from the Orfe at 0° C. Essentially the two tissues from each species show the same pattern of respiratory metabolism as a function of temperature, but the pattern differs as between species.

TABLE V

Q_{10} values calculated from data in Tables III and IV

Brain mince				Liver slice			
B. saida		*I. melanotus*		*B. saida*		*I. melanotus*	
Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}
0–5	1.78	0–2.5	4.57	0–5	1.84	0–2.5	3.33
5–10	2.16	2.5–5	2.09	5–10	2.09	2.5–5	5.32
10–15	2.30	5–7.5	4.82	10–15	2.36	5–7.5	4.57
15–20	2.02	7.5–10	9.99	15–20	2.14	7.5–10	3.39
20–25	—	10–15	2.78	20–25	2.02	10–15	2.53
		15–20	2.37			15–20	2.24
		20–25	2.19			20–25	2.06
Reg. line		Reg. line		Reg. line		Reg. line	
0–20	2.08	10–25	2.43	0–25	2.11	10–25	2.25

The Q_{10} values calculated from the data in Tables III and IV are given below in Table V, as well as the Q_{10} values obtained from the regression line over that portion of the curve that is rectilinear.

Discussion

The only other work which we may compare directly with our results is that of Fuhrman and co-workers (1944) on the metabolism of excised brain of the large mouthed bass, *Huro salmoides*. Figure 3 is taken from the results of these investigators.

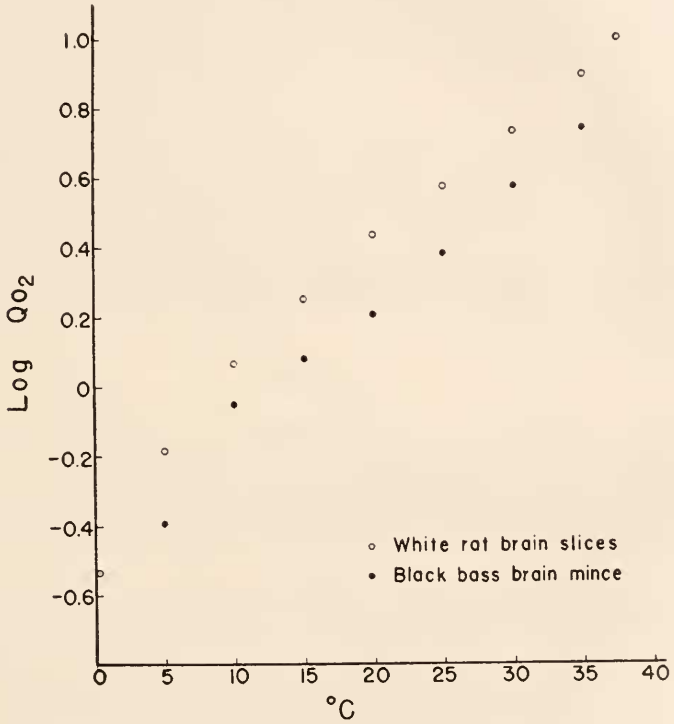

Figure 3. Logarithm of oxygen consumption of white rat brain slices (open circles) and black bass brain mince (solid circles) as a function of temperature.

It is apparent from inspection of this figure that the general pattern is similar to that of *I. melanotus* brain and liver. The Q_{10}'s, respectively, for the rat and bass brain over the range 10–35° C., are 2.14 and 2.06. In each case, over the range 0–10° C., there is an abrupt increase in Q_{10} to the order of 4–5. Thus, the only case we have found where Q_{10} remains essentially constant at the lower temperature is with brain and liver tissue from an arctic-adapted form, the polar cod.

Close inspection of Figures 1 and 2, in which log Q_{O_2} is plotted against temperature, reveals that the log Q_{O_2} may not truly be a rectilinear function of temperature. Other workers have pointed out examples where the rule of van't Hoff does

not adequately express the data. Wells (1935a) measured the respiratory metabolism of Fundulus at graded temperatures and found that the increased oxygen consumption with a rise in temperature was more pronounced at the lower temperatures. He concluded that the data could not be satisfactorily expressed either by the rule of van't Hoff or by the Arrhenius equation. For a discussion of temperature coefficients, see Belehradek (1930, 1935), Crozier (1924), Sizer (1943), Stearn (1949) and Wilson (1949). Similar results were obtained by Ege and Krogh (1915–16) in their studies on oxygen consumption of goldfish. These workers reported a Q_{10} of 9.8 for the range 0–5° C., 3.8 for the range 5–10° C., and over the range 10–28° C. the Q_{10} decreased from 2.9 to 2.2. Both cases, however, appear to follow the general pattern we have seen in tissues of *I. melanotus,* bass and albino rat, as do the results of Gasser (1931, 1933), working on temperature coefficients of spike potential, refractory period and conduction velocity in amphibian nerve.

The present observations show that the arctic cod has at least two advantages over the warm-adapted fish in adaptation to cold. First, at low temperatures the oxygen consumption per unit weight of brain and liver is several times as great; second, the temperature coefficient for oxygen consumption in brain and liver does not rise below 10° C. (as in the Orfe adapted to 25° C. and the bass adapted to 18° C.). Both the high Q_{O_2} and constant rather than increasing temperature coefficient at low temperatures are metabolic features of value in arctic adaptation.

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Summary

1. Respiratory metabolism has been studied in brain and liver tissues of two species of fish of similar mean body size, the arctic-adapted polar cod (*Boreogadus saida*), living at environmental temperatures of − 1.5 to + 2.0° C., and the Golden Orfe (*Idus melanotus*), living at an environmental temperature of 25° C. Experiments were carried out in the Warburg apparatus over the range 0–25° C.

2. Oxygen consumption in tissues of the Orfe was constant at all temperature levels studied for 180–240 minutes. In tissues of the polar cod, oxygen consumption was constant for 5–6 hours in the temperature range 0–5° C., and then the duration of the steady state decreased progressively as the temperature increased, so that at 25° C. constant oxygen consumption was observed in most cases for no longer than 40 minutes.

3. The Q_{10} for the steady state respiration of tissues of these two species of fish is of the same order of magnitude; values obtained were 2.08 and 2.11, respectively, for polar cod brain and liver, and 2.43 and 2.25 for Golden Orfe brain and liver. However, the Q_{10} remains essentially constant in the case of tissues of polar cod over the entire temperature range studied, whereas it increases sharply in Orfe tissues in the range 0–10° C. Thus, at low temperatures, the oxygen consumption of tissues of the cod, per unit weight, is several times as great as in the Orfe. These relationships are seen more strikingly when the logarithm of oxygen consumption is plotted against reciprocal of absolute temperature, or when the data are analyzed according to the Arrhenius equation.

4. These findings are discussed in relation to the problem of temperature adaptation in poikilotherms. It is concluded that the differences in response of tissues from the polar cod, compared with those of warm-adapted fish, are advantageous in adaptation of the organism to cold.

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